of discharging the patient, and two months after treatment respectively. As for INF- γ the levels were 37.6, 5.9, 0.18, 0.005 and for IL-12 the levels were 798.3, 865.2, 841.2 and 479 respectively.

Conclusion: We concluded that normalization of the plasma levels of INF- γ and IL-10 can serve as a reliable parameter in considering the patients as ''cured'' and determining the duration of the treatment.

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65.047

Detection of Acute *Toxoplasma gondii* infection in early pregnancy by IgG-avidity and PCR analysis

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Objective: To detect acute *Toxoplasma gondii* (*T. gondii*) infection in early pregnancy Introduction: Acute *Toxoplasma gondii* (*T. gondii*) infection in early pregnancy carries the risk of transmitting the infection to the fetus with serious sequelae. However, serological testing for IgG/IgM anti-Toxoplasma antibodies may fail to differentiate between a recent and past infection.

Methods: 224 Kuwaiti women in their first trimester were screened for IgG/IgM antibodies by Vitek Immuno Diagnostic Assay System (VIDAS) and VIDAS IgG-avidity tests.

Results & Discussion: On serological screening, 119 (53.1%) women were IgG-positive antibodies and 31 (13.8%) for IgM antibodies. Nine of the IgM-positive and 7 IgM-negative women had low avidity antibodies. However, IgG avidity test detected low avidity antibodies only in 9 (29%) of the 31 IgM-positive women suggesting a recent infection; and 19 (61.3%) women had high avidity antibodies indicating the infection was acquired in the distant past. Based on IgM serology alone, at least 31 IgM-positive women may have been wrongly labeled with acute Toxoplasma infection thus warranting appropriate therapeutic intervention. All the 19 IgM-positive women with high avidity were confirmed negative for Toxoplasma DNA on PCR analysis. Compared with PCR analysis the VIDAS avidity test was a helpful tool for the diagnosis of recent Toxoplasma infection in IgM-negative women with low-avidity and IgM-positive women with high avidity, specificity >85% to 100% respectively.

Conclusion: The VIDAS avidity test when used in combination with VIDAS IgG/IgM tests is a valuable assay for the exclusion of ongoing or recently acquired *T. gondii* infection in pregnant women in their first trimester and that it decrease significantly the necessity for follow-up testing and unnecessary therapeutic intervention.

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A 6-Year Geohelminth Infection Profile of Children at High Altitude in Western Nepal

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Background: Geohelminth infections are a major problem of children from the developing countries. Children with these infections suffer from developmental impairments and other serious illnesses. This study aimed to measure the prevalence of geohelminth infection, infection intensity as well as the change in the intensity in children from Western Nepal over years.

Methods: This 6-year hospital based prospective study at the Manipal Teaching Hospital, Pokhara included children (<15 years) visiting the hospital from Kaski and 7 surrounding districts. Samples were also collected from children in the community from different medical camps. Three stool samples from every child were processed using direct and concentration methods. The Kato-Katz technique was used for measuring the intensity of infection.

Results: The overall prevalence in hospital-attending children was 9.2% with 7.6% in preschool (0—5 y) and 11.0% in school-age (6—15 y). *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma deodenale* and *Strongyloides stercoralis* were the common geohelminths with a gradual decrease in worm load over the years. School-age children were found to be significantly more prone to geohelminth infection as compared to preschool children, but no statistical difference was detected by gender, district as well as season.

Conclusion: This heavy infection of geohelminths in children should be corrected by appropriate medication and maintaining strict personal hygiene. Health education, clean water, good sewage management and a congenial environment should be ensured to minimise infection.

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65.049

Production of Monoclonal Antibody Against *Toxocara cati* Second Stage Larvae and Its Application for the Detection of Circulating Antigens

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Background: Toxocariasis is a zoonotic disease caused by the migration or presence of *Toxocara canis* or *Toxocara cati* larva in human tissues or organs. Human become

infected by ingestion of soil or contaminated meat containing Toxocara larvae. Diagnosis of larval migrans is mainly based on detection of specific antibodies determined by enzyme-linked immunosorbent assay (ELISA), using larval excretory-secretory (ES) antigens. The aim of this study was to produce a monoclonal antibody against *Toxocara cati* second stage larvae antigens for possible immunodiagnosis of human toxocariasis.

Methods: Mice were immunized with *T. cati* L2S antigens in a 35 day course. Spleen cells from immunized mice were fused with P3U1 cells. Hybridoma cells were screened against *T. cati* larvae antigens, and positive antibody producing cells were selected and monoclonal antibody was purified from supernatant of hybridoma growing cells. The antibody was used in a sandwich ELISA system for detecting of *T. cati* antigen.

Results: The isotype of produced monoclonal antibody (TCTMAB) was IgG3. The produced monoclonal antibody reacted with *T. cati* larvae antigens while no cross reaction was found with antigens of both sexes of *Toxocara cati* adult worms, *Dirofilaria immitis*, *Ascaris sum* adult worm and metacercaria of *Centrocestus aromatus*. However the monoclonal antibody cross reacted with antigen of *Trichinella spiralis* larvae.

Conclusion: Our introduced sandwich ELISA seems to be a useful method for detection of *T. cati* antigen and could be used for proper serodiagnosis of human toxocariasis. The ELISA system had a sufficient sensitivity to detect 5 ng/ml of antigen.

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65.050

In Vitro Encystment of *Entamoeba histolytica* by Oxidative Stress and Oligoelements

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The inhibition of *Entamoeba histolytica* encystment represents a target to block the amoeba life cycle and impede its spreading. Unfortunately, the amoeba has not been encysted in vitro and drugs against the cyst are not available. Variation in the oxygen tension when the trophozoites move from the ascending to the descending colon could be within the luminal factors involved in the in vivo encystment. The formation of toxic reactive oxygen species in combination with other factors could activate cellular mechanisms of defense, including encystment. Therefore, we treated axenic cultures of HM1:IMSS trophozoites with a mix of hydrogen peroxide and several oligoelements (necessary for the activity of encystment enzymes) that resulted in the transformation of trophozoites to rounded and refringent-small structures which exhibited resistance to different detergents. Fluorescein diacetate treatment demonstrated that the viability of these cyst like-structures started to decrease after 6 hours post-induction, reaching 100% of death after 24 hours. Ultraestructural analysis by using scanning and transmission electron microscopy showed multinucleated structures (2, 3 and even, with 4 nuclei) with a smooth and thick membrane and multiple vacuoles. A decrease in the glycogen stores and a net of fibers similar to chitin was also observed. The presence of this polymer of N- acetilgalactosamine in the structures was demonstrated by intense blue fluorescence staining with white calcofluor as well as by positive lectures in a wheat germ agglutinin-based ELISA, two reagents that specifically binds to chitin. The expression of the *E. histolytica* Gln6Pi, rate-limiting enzyme in the chitin synthesis pathway, was evaluated by RT-PCR assay in untreated trophozoites and cyst-like structures. Results demonstrated the over-expression of this enzyme (6 folds) in the cyst-like structures, suggesting that the encystment route was activated in the trophozoites when exposed to our treatment.

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65.051

Evaluation of Lower Molecular Mass (10—30 KDa) *T. Solium cysticerci* antigen by Western blotting for the diagnosis of neurocysticercosis in children

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Background: Neurocysticercosis (NCC), the most common neurological disorder of parasite etiology results from lodgement of *T. Solium cysticerci* in the central nervous system. The confirmed diagnosis is based on radiological findings and serodiagnostic technique(s). The sensitivity and specificity of various serological techniques varies, depending upon the technique and antigen used. Lower molecular mass antigenic fraction of *T. Solium cysticerci* (Tso) have been found highly specific. Urine has been reported as better diagnostic sample than Serum in many microbial diseases due to advantage of collection by non-invasive method. The present study was aimed to evaluate LMM antigens for antibody detection in serum and urine samples by western blot for the diagnosis of NCC.

Methods: Serum and urine samples were collected from 125 clinically suspected and radiologically proven NCC patients and 125 control (60 other neurological diseases, 40 other parasitic diseases, 25 healthy) subjects. The Tso crude soluble extract antigen was prepared from naturally infected pigs and subjected to SDS-PAGE. Lower molecular Mass antigen (10—30 KDa) fraction was eluted and subjected to SDS-PAGE analysis followed by western blotting with the use of all the serum/urine samples.

Results: The analysis of western blot results by UVIPhotoMW software revealed four highly immunoreactive bands (24.7, 26.6, 28.8 & 30 KDa) with serum samples. Out of 125 NCC and 125 control serum samples, 24.7, 26.6, 28.8 & 30 KDa antigenic fractions were immunoreactive with 28.8%, 52%, 44%, 71.2% NCC samples and 8%, 20%, 16.8% & 27.2% control samples respectively. Out of 125 NCC and 125 control urine samples, 32% and 54.4% respectively were immunoreactive with only 30 KDa antigenic fraction.

Conclusion: The study suggests that 30KDa antigenic fraction appear to be the best diagnostic fraction for detection of antibody in serum samples and the urine sample may not